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Lipase catalysed kinetic resolutions of 3-aryl alkanolic acids.

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Abstract

Hydrolase catalysed kinetic resolutions leading to a series of 3-aryl alkanolic acids ($\geq 94\%$ ee) is described. Hydrolysis of the ethyl esters with a series of hydrolases was undertaken to identify biocatalysts that yield the corresponding acids with excellent enantiopurity in each case. Steric and electronic effects on the efficiency and enantioselectivity of the biocatalytic transformation was also explored.

Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are ubiquitous enzymes belonging to the family of serine hydrolases and are unequivocally, the most utilized enzymes in biocatalysis, providing one of the most advantageous and versatile biocatalytic methods in asymmetric synthesis.¹⁻¹¹ Hydrolases are excellent biocatalysts, combining wide substrate specificity with high regio- and enantioselectivity enabling the resolution of organic substrates with superb efficiency and selectivity.¹²⁻¹⁴ Furthermore, hydrolases do not require the use of labile co-factors, can be recycled, can be used in both free or immobilised form and are effective under mild, environmentally benign conditions and are biodegradable. These attributes make these catalysts especially attractive for the pharmaceutical and agrochemical areas, where the interest for enantiomerically pure and specifically functionalized compounds is continuously growing.^{2;4-11}

Hydrolase catalysed kinetic bioresolution is widely used to provide highly enantioenriched chiral carboxylic acids, which are valuable synthetic intermediates for the preparation of a variety of compounds of biological interest. There have been many reports on the successful resolution of 2-aryl or 2-aryloxy-propionic acids; the former are non-steroidal anti-inflammatory drugs and the latter an important class of herbicides.¹⁵⁻²⁰ Successful hydrolase catalysed resolution of alkanolic acids, with remotely located methyl-branching has been reported,^{21;22} however the literature has revealed limited success on the hydrolase mediated kinetic resolution of 3-aryl alkanolic acids. Enantiomerically pure 3-aryl alkanolic acids are used as chiral synthons in the asymmetric synthesis of antibacterial agents such as (-)-malyngolide, a naturally occurring δ -lactone of algae origin,²³ curcumene and curcuphenol, biological important bisabolene sesquiterpenes²⁴ and in the synthesis of amino acids β -methyl phenylalanine²⁵ and β -methyl tyrosine.²⁶ Within our own group, 3-aryl alkanolic acids are utilised in the synthesis of diazoketone derivatives, which in turn have been employed in Buchner cyclization reactions demonstrating excellent diastereoselectivity.^{27;28} This key transformation is currently under investigation in the efficient asymmetric synthesis of the bicyclo[5.3.0]decane skeleton, characteristic of daucane sesquiterpenoids.

Hydrolase catalysed non-aqueous enantioselective esterification of acids (\pm)-**1a**, (\pm)-**1b**, (\pm)-**1c** and (\pm)-**1d**, (Figure 1) has previously been reported, however substrate acids (\pm)-**1a** and (\pm)-**1b** were esterified with a modest to slow rate resulting in very low E values ($E < 2$) and no ester was observed under any conditions for acids (\pm)-**1c** and (\pm)-**1d**.²⁹ Traditional aqueous *Burkholderia cepacia* catalysed ester hydrolysis has been described for the resolution of 3-phenylbutanoic acid (\pm)-**1a** ($E > 50$), however this work has not been expanded to include acid substrates (\pm)-**1b**, (\pm)-**1c**, and (\pm)-**1d**, encompassing more sterically hindered substituents at the stereogenic centre.³⁰

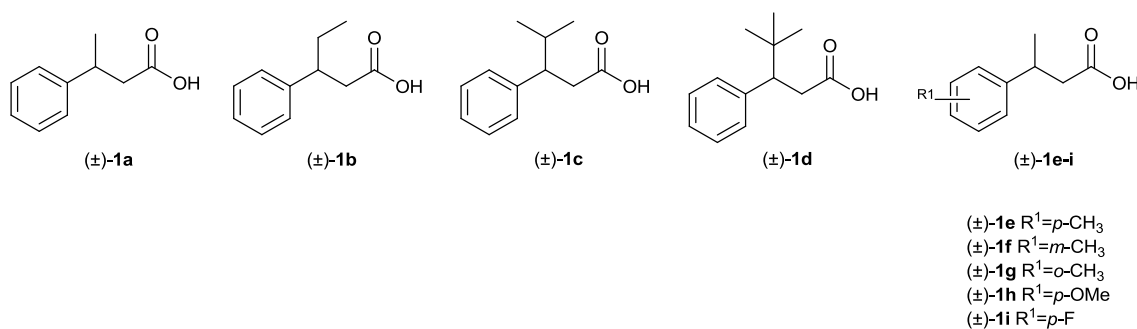


Figure 1.

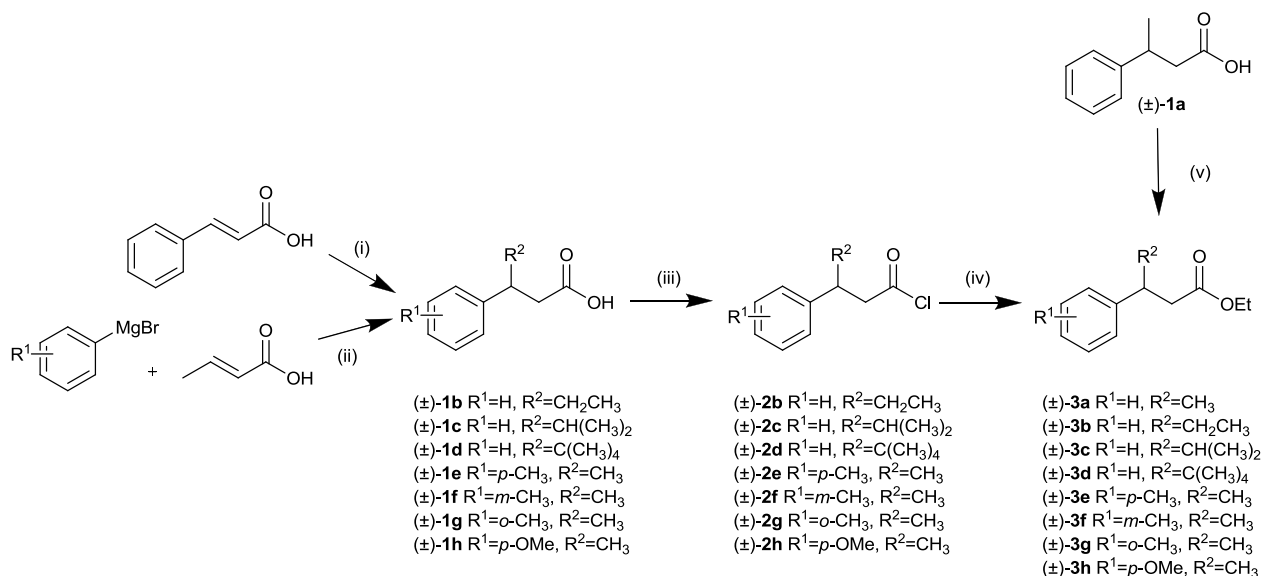
In this study, we wished to explore a wide range of hydrolases to establish if it is possible to generate the carboxylic acid (\pm)-**1a-i** in enantiopure form through kinetic bioresolution. Acids (\pm)-**1a-d**, were selected for investigation to determine the impact of steric effects at C3 on the efficiency of the kinetic resolution, while substrates (\pm)-**1e-i** were designed to explore both steric and electronic effects of substituents on the aromatic ring on the biotransformations. In contrast to the limited reported success in enantioselective esterification, this study focussed on enantioselective hydrolysis and indeed it was found that through appropriate choice of biocatalyst and reaction conditions, each of the carboxylic acids could be obtained in highly enantioenriched form.

Results and Discussion

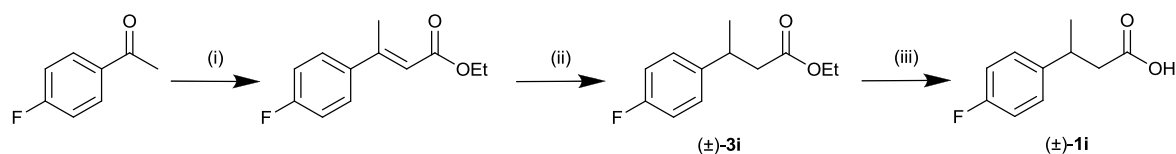
Synthesis of ethyl 3-aryl alkanoates.

Racemic ester (\pm)-**3a** was obtained *via* a simple Fischer esterification reaction from commercial 3-phenylbutanoic acid (\pm)-**1a** (Scheme 1). The 3-aryl alkanoic esters (\pm)-**3b-h** were synthesized in a three step synthesis. Acids (\pm)-**1b-d** were synthesised by conjugate addition of an alkyl Grignard to cinnamic acid, while acids (\pm)-**1e-h** were similarly prepared by conjugate addition of the appropriate aryl Grignard to crotonic acid.³¹ While direct esterification of the crude carboxylic acid was attempted, it was found that it was simpler to obtain the ethyl esters in analytically pure form by first transforming the carboxylic acid (\pm)-**1b-h** isolated from Grignard additions directly to the analogous acid chloride which was readily purified by vacuum distillation. Treatment of the pure acid chloride with ethanol in the presence of triethylamine led to analytically pure ester (Scheme 1). An alternative route *via* a Wadsworth-Emmons reaction was employed in synthesising ethyl 3-(4-fluorophenyl)butanoate (\pm)-**3i** (Scheme 2)³². Acids **1a-e** and **1h** have been previously

reported in the literature in enantioenriched form and therefore the assignment of absolute stereochemistry for each of these compounds was made by comparison of specific rotation data. Acids **1f-g** and **1i** have not been previously reported in enantiopure form and the absolute stereochemistry was determined in each case through crystallography studies.



Scheme 1. Synthesis of ethyl 3-aryl alkananoate (±)-**3a-h**. Reagents: (i) R^2MgX , Et_2O , (±)-**1b-d**; (ii) Et_2O , (±)-**1e-h**; (iii) $SOCl_2$; (iv) Et_3N , $EtOH$, CH_2Cl_2 ; (v) $EtOH$, cat. H_2SO_4 , (±)-**1a**.



Scheme 2. Synthesis of ethyl 3-(4-fluorophenyl)butanoate (±)-**3i** and ethyl 3-(4-fluorophenyl)butanoic acid (±)-**1i**. Reagents: (i) $(C_2H_5O)_2P(O)CH_2CO_2Et$, NaH , THF ; (ii) H_2 , Pd/C , $EtOH$; (iii) $NaOH$.³²

With racemic samples of both the esters and acids in hand, chiral HPLC conditions were developed for each ester hydrolysis in which both enantiomers of the ester and acid could be seen on a single trace (Figure 2). With a single injection, ready monitoring of both the efficiency and stereoselectivity of each of the hydrolase mediated transformations could be performed.

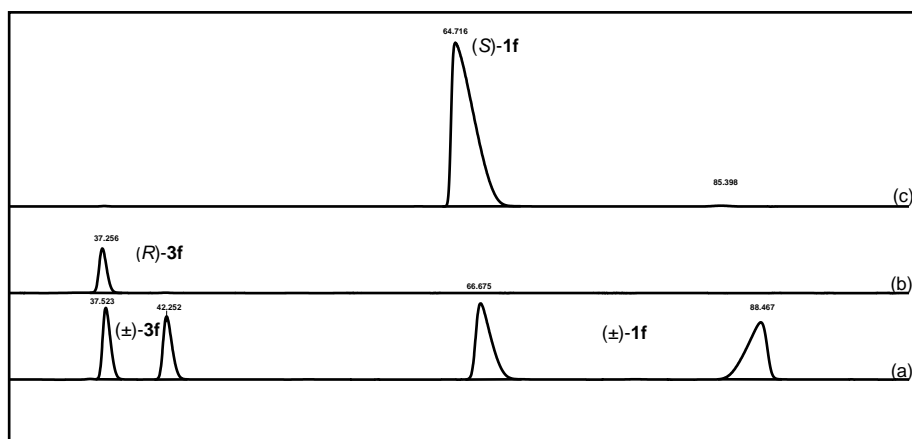


Figure 2: An overlay of HPLC traces of (a) A racemic mixture of ethyl 3-(3-methylphenyl)butanoic acid (\pm)-**1f** and ethyl 3-(3-methylphenyl)butanoate (\pm)-**3f**. (b) Enantiopure ethyl 3-(3-methylphenyl)butanoate (*R*)-**3f** and (c) Enantiopure 3-(3-methylphenyl)butanoic acid (*S*)-**1f**. See Table 7 for chiral HPLC conditions.

Hydrolase catalysed kinetic resolution of (±)-3-phenylbutanoic acid (±)-1a

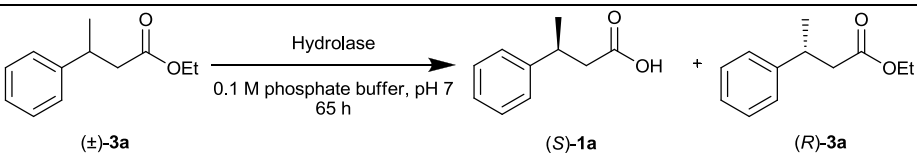
In total, 21 lipases and 1 esterase were screened in resolving racemic 3-phenylbutanoic acid (±)-1a. All of the hydrolases investigated resulted in hydrolysis of ethyl 3-phenylbutanoate (±)-3a to a certain extent and the screening results are summarised in Table 1. *Pseudomonas cepacia*, *Alcaligenes spp.* and *Pseudomonas fluorescens*, entry 6, 11 and 15 respectively, exhibited excellent enantioselection in the hydrolysis of substrate (±)-3a. *Burholderia cepacia* hydrolysis of the methyl ester of (±)-1a had previously been reported (E>50) providing access to the acid (S)-1a with 89% ee.³⁰ In this study, *Alcaligenes spp.* yielded the acid (S)-1a with excellent improved enantioselectivity of 97% ee (E>200) by hydrolysis of the corresponding ethyl ester (±)-3a. Unreacted (R)-3a was recovered in 98% ee providing access to both enantiomeric series in a single resolution.

From Table 1, it is evident that certain hydrolases hydrolysed the (R) enantiomer of substrate (±)-3a preferentially, providing access to the complementary enantiomer (R)-1a. *Candida antarctica* lipase B hydrolysis of (±)-3a had previously been reported to yield (R)-1a, (E=9),³³ while in this work, (Table 1, entry 16) it is clear that the (R) ester is selectively hydrolysed albeit at very low enantioselectivity. The less common pathway involving selective hydrolysis of the (R) ester has been successfully extended in this study to include the hydrolases *Candida Cyclindracea*, and *Mucor meihei*.³³

To demonstrate the practical viability of this process, *Pseudomonas fluorescens* was selected as the most appropriate hydrolase for preparative scale use. The conversion and enantiopurity of the esters and acids were analysed utilising chiral HPLC and after 64h, 50% conversion was achieved with 98% ee_{acid} and 99% ee_{ester} corresponding to an E value >200. Enantiopure samples of (S)-1a and (R)-3a were isolated by chromatography in 34% and 35% yield respectively. Column chromatography proved more effective than acid-base extraction for recovery of the ester and acid in this instance.

In practise, while use of *Candida antarctica* lipase B, *Candida Cyclindracea* and *Mucor meihei* all lead selectively to (R)-1a, access to the enantiopure (R)-1a is more effectively achieved through isolation of enantiopure (R)-3a using *Pseudomonas cepacia*, *Alcaligenes spp.* and *Pseudomonas fluorescens*, followed by saponification.

Table 1. Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-**3a**.

| <div style="text-align: center;">  </div> | | | | | | |
|---|--|-----------|---------------------|----------------|-----------------------------|----------------------|
| Entry | Enzyme | Temp (°C) | ee ^a (%) | | Conversion ^b (%) | E value ^b |
| | | | Ester 3a | Acid 1a | | |
| 1 | <i>Candida cyclindracea</i> C1 | 30 | 11 (S) | 58 (R) | 16 | 4.2 |
| 2 | <i>Candida cyclindracea</i> C2 | Ambient | 25 (S) | 59 (R) | 30 | 4.9 |
| 3 | <i>Rhizopus oryzae</i> | 30 | - | - | <10 ^c | - |
| 4 | <i>Achromobacter</i> spp. | 30 | 11 (R) | 90 (S) | 11 | 21 |
| 5 | <i>Alcaligenes</i> spp. 1 | 30 | 27 (R) | 95 (S) | 22 | 50 |
| 6 | <i>Pseudomonas cepacia</i> P1 | 30 | 99 (R) | 94 (S) | 51 | 170 |
| 7 | <i>Pseudomonas stutzeri</i> | Ambient | 14 (R) | 61 (S) | 19 | 4.7 |
| 8 | <i>Rhizopus</i> spp. | Ambient | - | - | <10 ^c | - |
| 9 | <i>Rhizopus niveus</i> | Ambient | - | - | <10 ^c | - |
| 10 | <i>Aspergillus niger</i> | Ambient | - | - | <10 ^c | - |
| 11 | <i>Alcaligenes</i> spp. 2 | Ambient | 98 (R) | 97 (S) | 50 | >200 |
| 12 | <i>Pseudomonas cepacia</i> P2 | Ambient | 96 (R) | 75 (S) | 56 | 26 |
| 13 | <i>Mucor javanicus</i> | Ambient | - | - | <10 ^c | - |
| 14 | <i>Penicillium camembertii</i> | Ambient | - | - | <10 ^c | - |
| 15 | <i>Pseudomonas fluorescens</i> | 30 | 99 (R) | 94 (S) | 51 | 170 |
| 16 | <i>Candida antarctica</i> lipase B | Ambient | 13 (S) | 0 (R) | 51 | 1.4 |
| 17 | <i>Mucor meihei</i> | Ambient | 3 (S) | 24 (R) | 11 | 1.7 |
| 18 | <i>Candida antarctica</i> lipase A | Ambient | 10 (R) | 68 (S) | 13 | 5.8 |
| 19 | <i>Candida antarctica</i> lipase B (immob) | Ambient | 70 (S) | 5 (R) | 93 | 1.8 |
| 20 | <i>Porcine pancrease</i> Type II | Ambient | 15 (R) | 93 (S) | 14 | 31 |
| 21 | <i>Porcine pancrease</i> Grade II | 30 | 35 (R) | 95 (S) | 27 | 54 |
| 22 | <i>Pig liver esterase</i> | Ambient | - | - | <10 ^c | - |

^aDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 95:5, flow rate 0.5 mL/min, 0 °C, λ=209.8 nm].

^bConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3a** (ee_s) and product acid **1a** (ee_p).³⁴

^cEstimated by chiral HPLC.

Hydrolase catalysed kinetic resolution of (±)-3-phenylpentanoic acid (±)-1b

The enzymatic hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**3b** proved to be significantly less facile than with (±)-**3a**. Of the 16 hydrolases screened, many displayed no catalytic activity for hydrolysis of the substrate ethyl 3-phenylpentanoate (±)-**3b**. Thus replacement of the methyl with the slightly larger ethyl moiety at the stereogenic centre C3 resulted in a very significant reduction of enzymatic activity. Just 6 of the hydrolases resulted in conversion as summarised in Table 2.

Significantly the biocatalysts which had yielded the most effective kinetic bioresolution with (±)-**3a** were ineffective for enzymatic hydrolysis of (±)-**3b**. For the 6 biocatalysts which resulted in ester hydrolysis the enantioselectivities were modest at best (Table 2). Interestingly with entries 2, 3 and 5 the poor enantioselectivity is associated with lack of discrimination of the enantiomers by the biocatalyst with conversions $\geq 78\%$ in each case, while the same biocatalysts with (±)-**3a** gave very limited reaction. The immobilised *Candida antarctica* lipase B provided the best results in this instance (E=25), this is in direct contrast to the limited reaction of (±)-**3a** with *Candida antarctica* lipase B (immob).

The direction of enantioselection in the hydrolysis of (±)-**3b** was consistent with that observed in the reactions of (±)-**3a** with *Candida antarctica* lipase B, *Candida antarctica* lipase B (immob) and *Pig liver esterase* providing the (*R*) acid selectively. Based on the initial promising result with *Candida antarctica* lipase B (immob) variation of the reaction conditions for the hydrolysis was undertaken to determine if the outcome could be optimised.

Table 2. Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**3b**.

| Entry | Enzyme ^a | Time | Temp (°C) | ee ^b (%) | | Conversion ^c (%) | E Value ^c |
|-------|------------------------------------|-------|--------------|---------------------|-------------------|--------------------------------|-------------------------|
| | | | | Ester 3b | Acid 1b | | |
| 1 | <i>Candida cyclindracea</i> C2 | 120 h | Ambient | - | - | <10 ^d | - |
| 2 | <i>Candida antarctica</i> lipase B | 65 h | Ambient | 80 (<i>S</i>) | 23 (<i>R</i>) | 78 | 3.4 |
| 3 | <i>Mucor meihei</i> | 67 h | Ambient | - ^e | - ^e | 100 | - ^e |
| 4 | <i>Candida antarctica</i> lipase A | 67 h | Ambient | 5 (<i>R</i>) | 44 (<i>S</i>) | 10 | 2.7 |
| 5 | <i>Pig liver esterase</i> | 65 h | Ambient | 87 (<i>S</i>) | 15 (<i>R</i>) | 85 | 3.1 |
| 6 | <i>Candida antarctica</i> | 65 h | Ambient | 85 (<i>S</i>) | 81 (<i>R</i>) | 51 | 25 |
| 7 | lipase B (immob) | 72 h | 4 | 62 (<i>S</i>) | 86 (<i>R</i>) | 42 | 24 |

^aThe following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes spp.* 2, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger* and *Mucor javanicus*.

^bDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 99:1, flow rate 0.5 mL/min, 0 °C, λ=209.8 nm].

^cConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3b** (ee_s) and product acid **1b** (ee_p).³⁴

^dEstimated by chiral HPLC.

^eReaction went to 100% completion, no enantioselectivity observed.

Temperature control in hydrolase-catalyzed resolutions has been explored due to its simplicity and reliability for enhancement of enantioselectivity, albeit at the expense of longer reaction times.^{7;35;36} Thus *Candida antarctica* lipase B (immob) resolution of (±)-**3b**, was performed at 4 °C, (Table 2, entry 7) resulting in a decrease in the extent of conversion even with an extended incubation period. No significant increase in enantioselection was observed, therefore this approach was not pursued further.

The utilisation of organic co-solvents has been well established to increase the enantioselectivity of hydrolase catalysed resolution of an extensive range of compounds.^{10;37;38} Screening reactions were therefore performed to assess the effect of a series of co-solvents (at 17% v/v) on *Candida antarctica* lipase B (immob) resolution of (±)-**3b** (Table 3). The majority of co-solvents investigated resulted in a decrease in the rate of hydrolysis, but notably, with the exception of TBME, resulted in an equivalent or an improved enantiopurity of (*R*)-**1b**. The utilisation of acetone as an additive, (Table 3, entry 3) resulted in recovery of (*R*)-**1b** with 94% ee and E=41 while with dioxane (Table 3, entry 5) E=51. Thus hydrolase catalysed resolution can be effective as a route to enantioenriched (*R*)-**1b** provided the biocatalyst and reaction conditions are carefully chosen. The only prior

report of hydrolase catalysed esterification of **1b** describes very low activity and enantioselectivity ($E < 2$).²⁹ Furthermore, the acid (*S*)-**1b** has been resolved using amidase biocatalysis and again enantiopurity was lower (88% ee).³⁹

Table 3. Investigation of co-solvent effect on *Candida antarctica* lipase B (immob) hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**3b**

| Entry | Co-solvent | Time | Temp (°C) | ee ^b (%) | | Conversion ^c (%) | E value ^c |
|-------|---------------------------|------|--------------|----------------------------------|---------------------------------|--------------------------------|-------------------------|
| | | | | Ester (<i>S</i>)- 3b | Acid (<i>R</i>)- 1b | | |
| 1 | DMSO | 64 h | Ambient | 93 | 81 | 53 | 31 |
| 2 | Acetonitrile ^a | 64 h | Ambient | 28 | 93 | 23 | 36 |
| 3 | Acetone ^a | 64 h | Ambient | 25 | 94 | 21 | 41 |
| 4 | THF | 64 h | Ambient | 6 | 88 | 6 | 16 |
| 5 | Dioxane | 64 h | Ambient | 72 | 92 ^d | 44 | 51 |
| 6 | TBME ^a | 64 h | Ambient | 24 | 57 | 30 | 4.6 |

^aHPLC grade

^bDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 99:1, flow rate 0.5 mL/min, 0 °C, λ =209.8 nm].

^cConversion and the enantiomeric ratio *E* was calculated from enantiomeric excess of substrate ester **3b** (ee_s) and product acid **1b** (ee_p).³⁴

^dOn one occasion the enantiomeric excess isolated from dioxane of (*R*)-**1b** was 97% ee.

Hydrolase catalysed kinetic resolution of (±)-4-methyl-3-phenylpentanoic acid (±)-**1c**

Given the decrease in biocatalytic activity on increasing the C3 substituent from methyl to ethyl it was anticipated that enzymatic hydrolysis to form (*S*)-**1c** and (*S*)-**1d** with the more sterically demanding *i*-propyl and *t*-butyl substituents would prove extremely challenging. Of the 19 hydrolases screened many displayed no hydrolytic activity towards (±)-**3c**, and hydrolysis failed to occur even at elevated temperature and extended reaction periods. Significantly, the hydrolases that were identified to hydrolyse (±)-**3b** were found to hydrolyse (±)-**3c** as depicted in Table 4 confirming that these biocatalysts can accommodate increased steric demand in the C3 region of the enzyme pocket.

Interestingly the extent of reaction in entries 1, 2 and 4, Table 4, is decreased somewhat relatively to those seen with (±)-**3b** in Table 2, resulting in improved enantiopurities of the recovered acid (*S*)-**1c**. Thus discrimination between the phenyl and *i*-propyl groups in the active site of the enzymes is improved somewhat relative to that seen in (±)-**3b** where discrimination between the ethyl and phenyl substituents is quite poor. While the *R*, *S* labels in the acid (*S*)-**1c** are switched relative to acids (*R*)-**1a** and (*R*)-**1b** the sense of enantioselection is identical in hydrolysis of the ethyl and *i*-propyl esters (±)-**3b** and (±)-**3c** with the *S* enantiomer isolated using enzymes *Candida antarctica* lipase B, *Mucor meihei* and *Candida antarctica* lipase B (immob). In this instance (*S*)-**1c** was obtained in 99% ee using *Candida antarctica* lipase B; hence no further optimisation was required.

Once again careful control of reaction conditions and selection of biocatalyst leads to efficient bioresolution of (*S*)-**1c**, in direct contrast to the literature report which states it was not possible to resolve this acid using hydrolase catalysis.²⁹

A solvent screen involving dioxane, acetone and TBME was conducted for *Candida antarctica* lipase A and *Candida antarctica* lipase B resolution of (\pm)-**3c** to investigate the effect on enantiomeric excess, but resulted in a significant reduction in activity and therefore was no longer pursued.

Table 4. Hydrolase-mediated hydrolysis of (\pm)-ethyl 4-methyl-3-phenylpentanoate (\pm)-**3c**.

| Entry | Enzyme ^a | ee ^b (%) | | Conversion ^c (%) | E value ^c |
|-------|---|---------------------|-----------------|--------------------------------|-------------------------|
| | | Ester 3c | Acid 1c | | |
| 1 | <i>Candida antarctica</i> lipase B | 12 (<i>R</i>) | 99 (<i>S</i>) | 11 | >200 |
| 2 | <i>Mucor meihei</i> | 61 (<i>R</i>) | 23 (<i>S</i>) | 73 | 2.7 |
| 3 | <i>Candida antarctica</i> lipase A | 10 (<i>S</i>) | 64 (<i>R</i>) | 14 | 5 |
| 4 | <i>Candida antarctica</i> lipase B (immob) | 33 (<i>R</i>) | 97 (<i>S</i>) | 25 | 90 |
| 5 | <i>Pig liver esterase</i> | - ^d | - ^d | 100 | - ^d |

^aThe following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes* spp. 1, *Penicillium camembertii*, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Candida cyclindracea* C2, *Rhizopus* spp., *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger*, *Alcaligenes* spp. 2 and *Mucor javanicus*

^bDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 98:2, flow rate 0.5 mL/min, 0 °C, λ =209.8 nm].

^cConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3c** (ee_s) and product acid **1c** (ee_p).³⁴

^dReaction went to 100% completion, no enantioselectivity observed.

Hydrolase catalysed kinetic resolution of (\pm)-4,4-dimethyl-3-phenylpentanoic acid (\pm)-**1d**

Hydrolase catalysed resolution of ethyl 4,4-dimethyl-3-phenylpentanoate (\pm)-**3d** was achieved using the same biocatalysts which catalysed reaction of (\pm)-**3b** and (\pm)-**3c** albeit at much lower extent of conversion presumably due to the increased steric demand of the C3 substituent. However, the overall trends are very similar for (\pm)-**3b**, (\pm)-**3c** and (\pm)-**3d** with the optimum results achieved with the immobilised or free *Candida antarctica* Lipase B, (Figure 3). While the extent of reaction at room temperature was extremely limited, increase of temperature improved the conversion somewhat, see for example entries 2 and 6, Table 5.

The direction of enantioselectivities is consistent with earlier observations for *Candida antarctica* lipase B (immob) and *Candida antarctica* lipase A. Interestingly the sense of enantioselection in the *Pig liver esterase* hydrolase, resulting in selective hydrolysis of the (*R*) enantiomer, is opposite to that seen in the hydrolysis of the corresponding ethyl derivative (\pm)-**3b**. Thus, in the ethyl derivative (\pm)-**3b** *Candida antarctica* lipase A provided the (*S*) enantiomer of the acid selectively while *Pig liver esterase* provides the (*R*) enantiomer selectively, whereas the *t*-butyl derivative *Pig liver esterase* displays the same direction of enantioselection as *Candida antarctica* lipase A.

With both the free and immobilised *Candida antarctica* lipase B, while the extent of the hydrolyse is limited the enantioselectivity is excellent, with the acid (*S*)-**1d** isolated in enantiopure form. Increasing the temperature improved the conversion somewhat, thereby resulting in an increased enantiopurity of the unreacted ester (*R*)-**3d**.

Table 5. Hydrolase-mediated hydrolysis of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**3d** at variable temperature.

| Entry | Enzyme ^a | Temperature °C | ee ^e (%) | | Conversion ^f (%) | E value ^f |
|-------|--|----------------------------|---------------------|-------------------------------|--------------------------------|----------------------|
| | | | Ester 3d | Acid 1d | | |
| 1 | <i>Candida</i> | Ambient ^b | 2 (<i>R</i>) | ≥99 ^h (<i>S</i>) | 2 | >200 |
| 2 | <i>antarctica</i> lipase B | 35 °C – 40 °C ^c | 23 (<i>R</i>) | ≥99 ^h (<i>S</i>) | 19 | >200 |
| 3 | <i>Candida</i> | Ambient ^b | 3 (<i>S</i>) | 73 (<i>R</i>) | 4 | 6.6 |
| 4 | <i>antarctica</i> lipase A | 35 °C – 40 °C ^d | 7 (<i>S</i>) | 81 (<i>R</i>) | 8 | 10 |
| 5 | <i>Candida</i> | Ambient ^b | 1 (<i>R</i>) | ≥99 ^h (<i>S</i>) | 1 | >200 |
| 6 | <i>antarctica</i> lipase B (immob) | 35 °C – 40 °C ^c | 30 (<i>R</i>) | 98 (<i>S</i>) | 23 | 132 |
| 7 | <i>Pig liver</i> | Ambient ^b | 32 (<i>S</i>) | 34 (<i>R</i>) | 48 | 2.7 |
| 8 | <i>esterase</i> | 35 °C – 40 °C ^c | - ^g | - ^g | 100 | - ^g |

^aThe following hydrolases gave no conversion *Pseudomonas cepacia* P1, *Rhizopus niveus*, *Pseudomonas fluorescens*, *Candida cyclindracea* C1, *Pseudomonas cepacia* P2 and *Porcine Pancrease* Type II.

^bTime for ester hydrolysis was 66 h.

^cTime for ester hydrolysis was 64.5 h at 35 °C temperature increased to 40 °C for the final 24 h.

^dTime for ester hydrolysis was 72 h at 35 °C temperature increased to 40 °C for the final 24 h.

^eDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 96:4, flow rate 0.25 mL/min, 20 °C, λ=209.8 nm].

^fConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3d** (ee_s) and product acid **1d** (ee_p).³⁴

^gReaction went to 100% completion, no enantioselectivity observed.

^hWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

It is evident that once the alkyl group at the C3 stereogenic centre increases in size greater than a methyl substituent, a dramatic decrease in the efficiency of the hydrolysis and thereby the kinetic bioresolution with regards to the enantiopurity of the ester is observed. Despite the steric hindrance within the active site, 3-aryl alkanoic carboxylic acids **1a-d** can be obtained through optimisation of reaction conditions with excellent enantioselectivity. The acid (*S*)-**1a** was obtained in 98% ee, through *Pseudomonas fluorescens* catalysed hydrolysis of (±)-**3a**,

while acids (*R*)-**1b**, (*S*)-**1c** and (*S*)-**1d** were obtained in $\geq 94\%$ ee *via* immobilised or free *Candida antarctica* lipase B catalysed kinetic bioresolution (Figure 3).

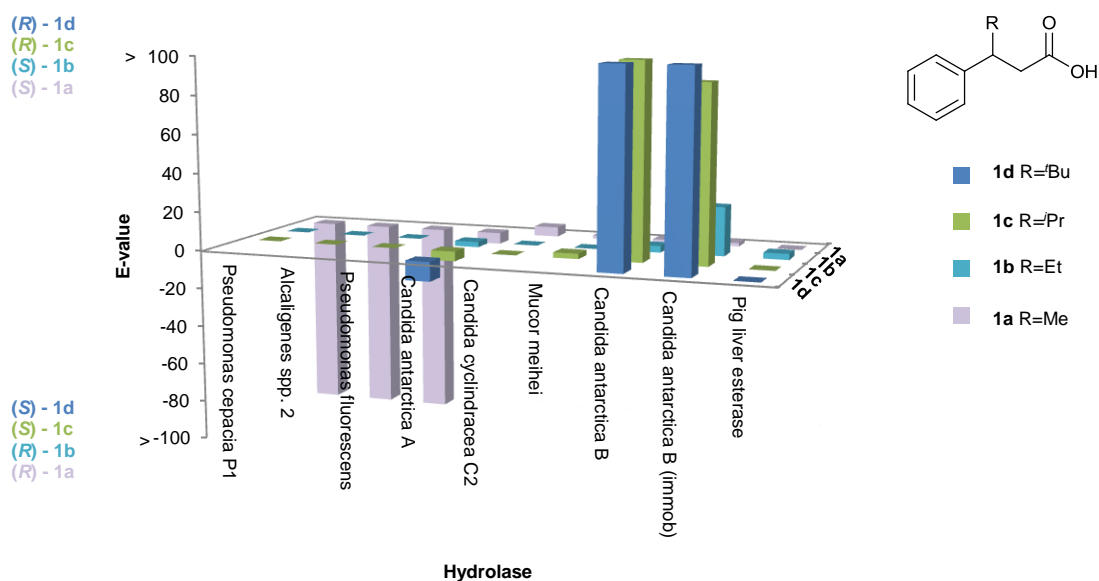


Figure 3. Graph of enantiomeric ratio (E) versus hydrolase – Kinetic bioresolutions for **1a-d** performed under standard aqueous conditions.

Significantly, *Candida antarctica* lipase A provides a viable route to the complementary enantiomers (*S*)-**1b** and (*R*)-**1c-d** overcoming the limitation of the modest enantiomeric excess of the esters achieved in the resolutions using the free and immobilised *Candida antarctica* lipase B. In addition, *Candida antarctica* lipase A has a unique ability to accept very bulky, highly sterically hindered substrates and this correlated with the observations of this study where the enantiopurity of the acid obtained *via* *Candida antarctica* lipase A catalysed resolution improved as the size of the alkyl substituent at C3 increased, the highest enantiopurity obtained being of (*R*)-**1d** at 81% ee (Table 5, entry 4).^{40;41}

Hydrolase catalysed kinetic resolution of substituted phenyl butanoic acids (\pm)-**1e-i**

A series of substituted phenyl butanoic acids were selected for investigation enabling exploration of the impact of substituents on the aryl ring on the efficiency of the kinetic bioresolution process. The substrates selected were *ortho*-, *meta*- and *para*-tolylbutanoic acid and *para*-methoxy, *para*-fluoro-phenyl butanoic acids enabling exploration of the electronic effect in addition to the impact of the position of substitution, (Figure 4).

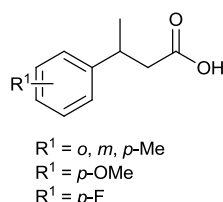


Figure 4.

The outcome of the enzymatic screens are summarised in Tables 6-10. In each case effective kinetic bioresolution was achieved with *Pseudomonas cepacia* P1, *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* resulting in successful hydrolysis of the *S* enantiomer with very similar outcomes to those seen with 3-phenylbutanoic acid (*S*)-**1a** indicating that the aryl substituent had little impact on the enzymatic hydrolysis.

Table 6. Hydrolase-mediated hydrolysis of (\pm)-ethyl 3-(4-methylphenyl)butanoate (\pm)-**3e**

| Entry | Enzyme | Temp (°C) | ee ^a (%) | | Conversion ^b (%) | E value ^b |
|-------|---|--------------|-------------------------------|-----------------|--------------------------------|-------------------------|
| | | | Ester 3e | Acid 1e | | |
| 1 | <i>Pseudomonas cepacia</i> P1 | 30 | 98 (<i>R</i>) | 99 (<i>S</i>) | 50 | >200 |
| 2 | <i>Pseudomonas cepacia</i> P2 | 30 | 99 (<i>R</i>) | 96 (<i>S</i>) | 51 | >200 |
| 3 | <i>Pseudomonas fluorescens</i> | 30 | ≥99 ^d (<i>R</i>) | 95 (<i>S</i>) | 51 | >200 |
| 4 | <i>Candida cyclindracea</i> | 30 | - ^c | - ^c | 0 | - ^c |
| 5 | <i>Candida antarctica</i> lipase A | 30 | 5 (<i>R</i>) | 68 (<i>S</i>) | 7 | 5.5 |
| 6 | <i>Candida antarctica</i> lipase B (immob) | 30 | 6 (<i>S</i>) | 5 (<i>R</i>) | 55 | 1.2 |

^aDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 99.5:0.5, flow rate 0.5 mL/min, 0 °C, λ=211 nm].

^bConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3e** (ee_s) and product acid **1e** (ee_p).³⁴

^cReaction failed to proceed, no enantioselectivity observed.

^dWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

Table 7. Hydrolase-mediated hydrolysis of (±)-ethyl 3-(3-methylphenyl)butanoate (±)-**3f**

| Entry | Enzyme | Temp (°C) | ee ^{a,b} (%) | | Conversion ^c (%) | E value ^c |
|-------|---|--------------|-------------------------------|-----------------|--------------------------------|-------------------------|
| | | | Ester 3f | Acid 1f | | |
| 1 | <i>Pseudomonas cepacia</i> P1 | 30 | 88 (<i>R</i>) | 96 (<i>S</i>) | 48 | 143 |
| 2 | <i>Pseudomonas cepacia</i> P2 | 30 | ≥99 ^e (<i>R</i>) | 76 (<i>S</i>) | 57 | 52 |
| 3 | <i>Pseudomonas fluorescens</i> | 30 | 96 (<i>R</i>) | 97 (<i>S</i>) | 50 | >200 |
| 4 | <i>Candida cyclindracea</i> | 30 | - ^d | - ^d | 0 | - ^d |
| 5 | <i>Candida antarctica</i> Lipase B (immob) | 30 | ≥99 ^e (<i>S</i>) | 7 (<i>R</i>) | 93 | 4.7 |

^aTime for ester hydrolysis was 65 h with the exception of *Candida cyclindracea* catalysed hydrolysis which was 64 h.

^bDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 98:2, flow rate 0.25 mL/min, 0 °C, λ=209.8 nm].

^cConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3f** (ee_s) and product acid **1f** (ee_p).³⁴

^dReaction failed to proceed, no enantioselectivity observed.

^eWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

Table 8. Hydrolase-mediated hydrolysis of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-**3g**

| Entry | Enzyme Source | Temp (°C) | ee ^{a,b} (%) | | Conversion ^c (%) | E value ^c |
|-------|---|--------------|-------------------------------|-------------------------------|--------------------------------|-------------------------|
| | | | Ester 3g | Acid 1g | | |
| 1 | <i>Pseudomonas cepacia</i> P1 | 30 | ≥99 ^e (<i>R</i>) | 99 (<i>S</i>) | 50 | >200 |
| 2 | <i>Pseudomonas cepacia</i> P2 | 30 | ≥99 ^e (<i>R</i>) | 80 (<i>S</i>) | 56 | 65 |
| 3 | <i>Pseudomonas fluorescens</i> | 30 | ≥99 ^e (<i>R</i>) | ≥99 ^e (<i>S</i>) | 50 | >200 |
| 4 | <i>Candida cylindracea</i> | 30 | - ^d | - ^d | 0 | - ^d |
| 5 | <i>Candida antarctica</i> Lipase B (immob) | 30 | 90 (<i>R</i>) | 46 (<i>S</i>) | 66 | 7.7 |

^aTime for ester hydrolysis was 67 h with the exception of *Pseudomonas fluorescens* catalysed hydrolysis which was 64 h.

^bDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 94:6, flow rate 0.25 mL/min, 0 °C, λ=209.8 nm].

^cConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3g** (ee_s) and product acid **1g** (ee_p).³⁴

^dReaction failed to proceed, no enantioselectivity observed.

^eWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

Table 9. Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-**3h**

| Entry | Enzyme | Temp (°C) | ee ^a (%) | | Conversion ^b (%) | E value ^b |
|-------|---|--------------|-------------------------------|-----------------|--------------------------------|-------------------------|
| | | | Ester 3h | Acid 1h | | |
| 1 | <i>Pseudomonas cepacia</i> P1 | 30 | 98 (<i>R</i>) | 86 (<i>S</i>) | 53 | 60 |
| 2 | <i>Pseudomonas cepacia</i> P2 | 30 | 99 (<i>R</i>) | 88 (<i>S</i>) | 53 | 81 |
| 3 | <i>Pseudomonas fluorescens</i> | 30 | ≥99 ^d (<i>R</i>) | 97 (<i>S</i>) | 51 | >200 |
| 4 | <i>Candida Antarctica</i> Lipase A | 30 | 4 (<i>R</i>) | 48 (<i>S</i>) | 8 | 3 |
| 5 | <i>Candida cylindracea</i> | 30 | - | - | <10 ^c | - |
| 6 | <i>Candida antarctica</i> Lipase B (immob) | 30 | 66 (<i>S</i>) | 7 (<i>R</i>) | 90 | 1.9 |

^aDetermined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 82:18, flow rate 0.25 mL/min, 0 °C, λ=216.9 nm].

^bConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3h** (ee_s) and product acid **1h** (ee_p).³⁴

^cEstimated by chiral HPLC.

^dWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

Table 10. Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-**3i**

| Entry | Enzyme | Temp (°C) | ee ^a (%) | | Conversion ^b (%) | E value ^b |
|-------|---|--------------|----------------------|----------------|--------------------------------|-------------------------|
| | | | Ester 3i | Acid 1i | | |
| 1 | <i>Pseudomonas cepacia</i> P1 | 30 | ≥99 ^d (R) | 84 (S) | 54 | 59 |
| 2 | <i>Pseudomonas cepacia</i> P2 | 30 | ≥99 ^d (R) | 69 (S) | 84 | 27 |
| 3 | <i>Pseudomonas fluorescens</i> | 30 | ≥99 ^d (R) | 94 (S) | 62 | 170 |
| 4 | <i>Candida cyclindracea</i> | 30 | 3 (S) | 25 (R) | 11 | 1.7 |
| 5 | <i>Candida antarctica</i> Lipase B (immob) | 30 | - ^c | - ^c | 100 | - ^c |

^aDetermined by chiral HPLC analysis [Daicel Chiralcel AS-H column, Step gradient: 0 °C, λ=256 nm, hexane/*i*PrOH (3% trifluoroacetic acid), 0-30 min; 99.7:0.3, flow rate 1 mL/min. 31 min; 94:6, flow rate 0.25 mL/min].

^bConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3i** (ee_s) and product acid **1i** (ee_p).³⁴

^cReaction went to 100% completion, no enantioselectivity observed.

^dWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

In all cases highly enantioenriched samples of the 3*S* acids and the 3*R* esters are readily obtained using the *Pseudomonas* biocatalysts, see Figure 5. 3-(4-Methylphenyl)butanoic acid (±)-**1e** had previously been resolved utilising *Pseudomonas cepacia* immobilized on ceramic particles to yield (*S*)-**1e** in 99% ee.²⁴ The results obtained in this study utilising the free hydrolase, Table 6, entry 1, correlate strongly. The only significant effect of substituent seen in this series of substituted phenyl butanoic acids was with the *para*-F substrate (±)-**3i** where the conversion is increased relative to the other substrates resulting in a slight decrease in enantiopurity of the recovered acids. Use of the *Candida cyclindracea* biocatalyst with the substituted substrates was also explored; while *Candida cyclindracea* had resulted in some hydrolysis with the parent compound (±)-**3a**, very little conversion was seen with the substituted derivatives (Figure 5).

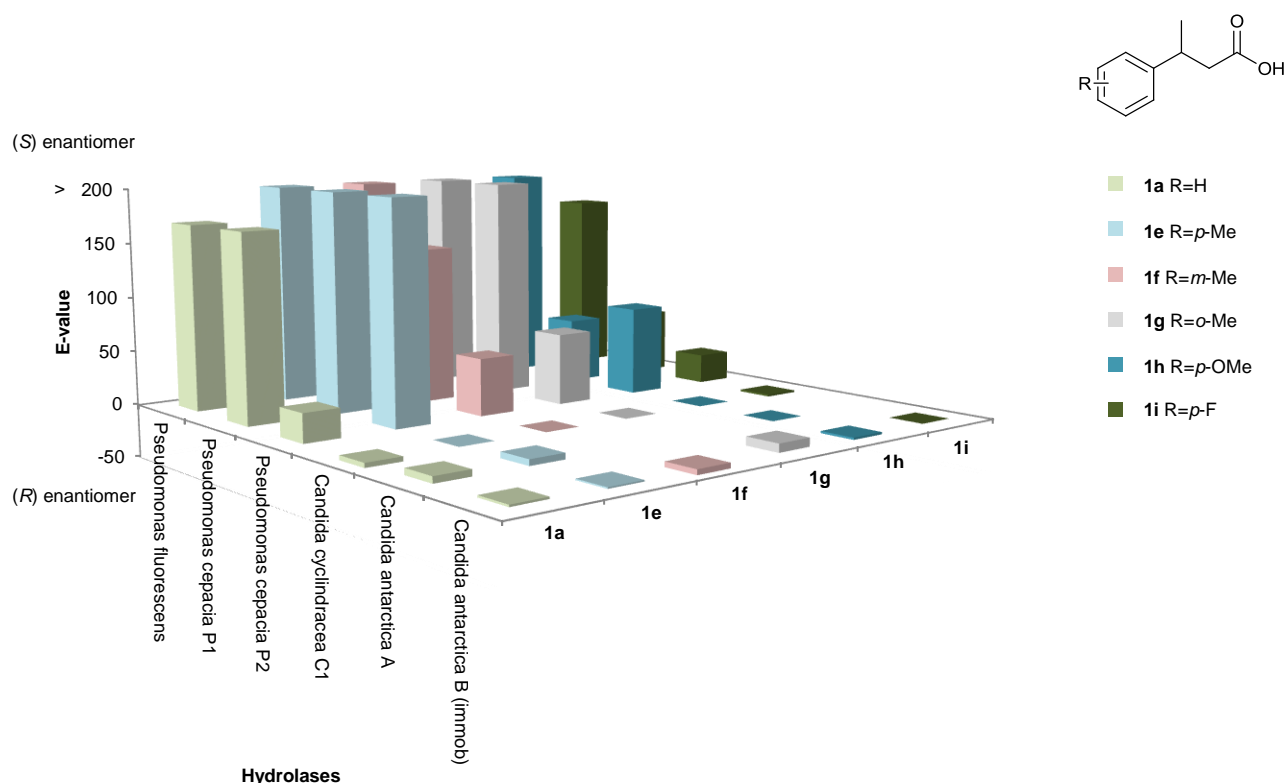


Figure 5. Graph of enantiomeric ratio (E) versus hydrolase - kinetic bioresolutions for **1a** and **1e-i** performed under standard aqueous conditions.

Use of *Candida antarctica* Lipase B (immob) with the parent substrate (\pm)-**1a** provided access to the opposite enantiomeric series (Table 1, entry 19), albeit with a very high extent of reaction. In general similar reaction patterns were seen with the substituted substrates resulting in recovery of the (*S*)-esters **3e-f** and **3h** and the (*R*)-acids **1e-f** and **1h**. Notably, with the *p*-Me substrate (\pm)-**3e** the extent of reaction was less, resulting in a decrease in enantiopurity of the recovered ester (*S*)-**3e**, while with the *para*-F substrate both enantiomers are completely indiscriminately hydrolysed. Uniquely, the sense of enantioselection in *Candida antarctica* lipase B (immob) resolution of the *o*-Me substrate (\pm)-**3g** was the same as that seen with the *Pseudomonas* biocatalysts. Interestingly this biocatalyst was the one that was able to accommodate increased steric demand at the C3 position. The switch in enantioselection must be due to combined steric effects of the *ortho*-Me and 3-Me substituents possibly *via* conformational changes, (Figure 6).

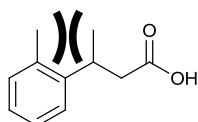
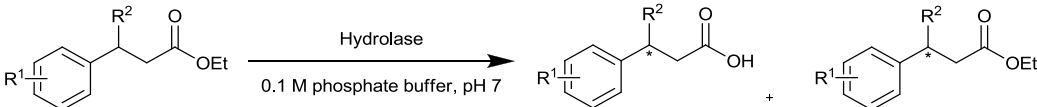


Figure 6.

As summarised in Table 11, nine of the hydrolyse were scaled up to synthetic batches leading to isolations of acids **1a-i** and esters **3a-i** in excellent enantiopurity in most cases. Significantly, on scale up, the efficiencies and selectivities mirrored quite closely the outcomes seen in the analytical scale reactions summarised in Tables 1-10. Notably with the

para-F series the acid (*S*)-**1i** was recovered in excellent enantiopurity in contrast to the small scale reaction (Table 10, entry 3). With the 3-aryl butanoic acids the products of the biocatalysis were readily isolated by extraction with ethyl acetate followed by chromatographic separation of the acids and esters. With the bulkier 3-alkyl substituents, chromatography was less effective. In practice the ester was recovered first by heptane extraction of the biotransformation mixture, then subsequent acidification and extraction with ethyl acetate provided the acids (*R*)-**1b** and (*S*)-**1c-d**. Each of the products were characterised by ¹H NMR and chiral HPLC.

Table 11. Synthetic scales of hydrolase-mediated hydrolysis of (±)-**3a-i**

|  | | | | | | | | |
|--|-----------------|----------------------------|-----------------------------------|---|---|---|-----------------------------|----------------------|
| Entry | Ester substrate | | | Enzyme | ee ^a (%) | | Conversion ^b (%) | E value ^b |
| | | R ¹ | R ² | | Ester | Acid | | |
| 1 | 3a | H | CH ₃ | <i>Pseudomonas fluorescens</i> | 99 (<i>R</i>)- 3a | 98 (<i>S</i>)- 1a | 50 | >200 |
| 2 | 3b | H | CH ₂ CH ₃ | <i>Candida antarctica</i> lipase B (immob) ^c | 65 (<i>S</i>)- 3b | 90 (<i>R</i>)- 1b | 42 | 37 |
| 3 | 3c | H | CH(CH ₃) ₂ | <i>Candida antarctica</i> lipase B (immob) | 26 (<i>R</i>)- 3c | 98 (<i>S</i>)- 1c | 21 | 127 |
| 4 | 3d | H | C(CH ₃) ₃ | <i>Candida antarctica</i> lipase B (immob) | 12 (<i>R</i>)- 3d | 99 (<i>S</i>)- 1d | 11 | >200 |
| 5 | 3e | <i>p</i> -CH ₃ | CH ₃ | <i>Pseudomonas cepacia</i> P1 | 97 (<i>R</i>)- 3e | ≥99 ^d (<i>S</i>)- 1e | 49 | >200 |
| 6 | 3f | <i>m</i> -CH ₃ | CH ₃ | <i>Pseudomonas fluorescens</i> | 94 (<i>R</i>)- 3f | ≥99 ^d (<i>S</i>)- 1f | 49 | >200 |
| 7 | 3g | <i>o</i> -CH ₃ | CH ₃ | <i>Pseudomonas fluorescens</i> | 98 (<i>R</i>)- 3g | ≥99 ^d (<i>S</i>)- 1g | 50 | >200 |
| 8 | 3h | <i>p</i> -OCH ₃ | CH ₃ | <i>Pseudomonas fluorescens</i> | 99 (<i>R</i>)- 3h | 97 (<i>S</i>)- 1h | 51 | >200 |
| 9 | 3i | <i>p</i> -F | CH ₃ | <i>Pseudomonas fluorescens</i> | ≥99 ^d (<i>R</i>)- 3i | 97 (<i>S</i>)- 3i | 51 | >200 |

^aFor chiral HPLC conditions see details on relevant Tables 1-10.^bConversion and the enantiomeric ratio E was calculated from enantiomeric excess of substrate ester **3a-i** (ee_s) and product acid **1a-i** (ee_p).³⁴^c17% v/v dioxane co-solvent was added, see experimental for further information.^dWhen the second enantiomer is not observed enantiomeric excess is stated as ≥99% ee.

Conclusion

In this study, a series of 3-aryl alkanolic acids (\pm)-**1a-i** were successfully resolved with enantiopurity $\geq 94\%$ ee *via* hydrolase catalysed kinetic hydrolysis of the corresponding ethyl esters. It was apparent upon resolving acids (\pm)-**1a-d** that a large reduction in reaction rate and enantioselectivity was observed once the moiety at the C3 stereogenic centre increased in size greater than a methyl. Despite this, the highest obtained enantiopurities of hydrolase catalysed bioresolutions of 3-aryl alkanolic acids (\pm)-**1a-d** are reported through optimisation of reaction conditions and a viable route to both enantiomers has been identified. Furthermore, substituents on the phenyl ring, acids (\pm)-**1e-i** were determined to have limited effect on the excellent enantioselectivities attainable concluding that the hydrolases can tolerate increased steric demand in the aryl group more readily than in the 3-alkyl group. Significantly nine of these experiments were conducted on a synthetic scale leading to isolation of acids **1a-i** and esters **3a-i** in excellent enantiopurity in most cases.

Experimental

All solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorus pentoxide and ethyl acetate was distilled from potassium carbonate. Ethanol was distilled from magnesium in the presence of iodine and stored over 3 Å molecular sieves. Hexane was distilled prior to use. Tetrahydrofuran was distilled from sodium and benzophenone. Molecular sieves were activated by heating at 150 °C overnight. Organic phases were dried using anhydrous magnesium sulphate. Infrared spectra were recorded as thin films on sodium chloride plates for oils or as potassium bromide (KBr) discs for solids on a Perkin Elmer Paragon 1000 FT-IR spectrometer.

^1H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. ^1H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. All spectra were recorded at room temperature (~20 °C) in deuterated chloroform (CDCl_3) unless otherwise stated using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ_{H} & δ_{C}) are reported in parts per million (ppm) relative to TMS and coupling constants are expressed in Hertz (Hz).

Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluant; samples were made up in acetonitrile. High resolution mass spectra (HRMS) were recorded on a Waters LCT premier Time of Flight spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluant; samples were made up in acetonitrile.

Elemental analysis were performed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers. Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus and are uncorrected. Wet flash chromatography was performed using Kieselgel silica gel 60, 0.040-0.063 mm (Merck). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF₂₅₄). Visualisation was achieved by UV (254nm) light detection and bromocresol green staining.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 589 nm in a 10 cm cell; concentrations (c) are expressed in g/100 mL. $[\alpha]_D^{20}$ is the specific rotation of a compound and is expressed in units of $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. The hydrolases used for these biotransformations were obtained from Almac Sciences. All reagents are analytical grade and purchased from Sigma Aldrich chemical company. All enzymatic reactions were performed on a VWR Incubating Mini Shaker 4450. Enantiomeric purity of acids **1a-h** and esters **3a-h** were determined by chiral HPLC analysis on a Chiralcel OJ-H column (5×250 mm), enantiomeric purity of acid **1i** and ester **3i** were determined on a Chiralcel AS-H column (5×250 mm) purchased from Daicel Chemical Industries, Japan. Mobile phase, flow rate, detection wavelength and temperature are stated in the appropriate Table 1-10. HPLC analysis was performed on a Waters alliance 2690 separations module with a PDA detector. All solvents employed were of HPLC grade. Bulb to bulb distillations were carried out on an

Aldrich Kugelrohr apparatus and the oven temperature is given as the boiling point of the substrate. (±)-3-(*p*-Fluorophenyl)butanoic acid (±)-**1i** and (±)-ethyl 3-(*p*-fluorophenyl)butanoate (±)-**3i** were prepared according to the procedure described by Speranza.³²

Synthesis of Carboxylic Acids

(±)-3-Phenylpentanoic acid (±)-**1b**^{28;31;42}

Ethyl magnesium bromide was freshly prepared from magnesium (2.85 g, 117 mmol) and iodine (catalytic amount) in diethyl ether (30 mL) and ethyl bromide (8.7 mL, 117 mmol) in diethyl ether (30 mL) at 0 °C under nitrogen and the mixture was stirred for 0.5 h at 0 °C. Cinnamic acid (5.00 g, 33.75 mmol) was added portionwise while stirring at 0 °C, then the reaction mixture was heated under reflux for 3 h. The reaction mixture was subsequently cooled to room temperature and carefully poured onto aqueous hydrochloric acid (10%, ~100 mL) and ice (~65 g). The layers were separated and the aqueous layer was washed with diethyl ether (3 × 50 mL). The combined organic layer was washed with hydrochloric acid (10%, 50 mL), water (50 mL), brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude acid (±)-**1b** (5.54 g, 92%) as an orange oil which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2965 (OH), 1708 (CO), 1603, 1495, 1454; δ_{H} (400 MHz) 0.79 [3H, t, J 7.4, C(5)H₃], 1.46–1.83 [2H, m, C(4)H₂], 2.60 [1H, dd, A of ABX, J_{AB} 15.6, J_{AX} 7.9, one of C(2)H₂], 2.68 [1H, dd, B of ABX, J_{AB} 15.6, J_{BX} 7.1, one of C(2)H₂], 2.89–3.08 [1H, m, X of ABX, C(3)H], 7.09–7.38 (5H, m, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

Aqueous potassium hydroxide (20%, 35 ml) was added to a sample of 3-phenylpentanoyl chloride (±)-**2b** (0.5 g, 2.6 mmol) under nitrogen. The reaction mixture was heated to reflux and stirred under reflux overnight. The reaction mixture was then cooled to room temperature and acidified to pH 1 with aqueous hydrochloric acid (10%), which resulted in a white precipitate. The suspension was then extracted with dichloromethane (3 × 50 ml), and the combined organic layers were washed with brine (100 ml), dried, filtered and concentrated under reduced pressure to give the acid (±)-**1b** (0.428 g, 92%) as a cream solid, m.p. 59–62 °C (Lit.,³¹ 60–61 °C) and with spectral characteristics identical to those described above.

(±)-4-Methyl-3-phenylpentanoic acid (±)-**1c**^{28;42}

This was prepared following the procedure described for (±)-**1b** from isopropyl magnesium bromide [freshly prepared from magnesium (2.70 g, 111 mmol) and iodine (catalytic amount) in diethyl ether (30 mL), and isopropyl bromide (11.25 mL, 120 mmol) in diethyl ether (30 mL)] and cinnamic acid (5.00 g, 33.75 mmol) to give the crude acid (±)-**1c** (5.38 g, 83%) as an orange oil, which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2963 (OH), 1709 (CO), 1602, 1495, 1454; δ_{H} (400 MHz) 0.74, 0.92 [2 × 3H, 2 × d, J 6.7, J 6.7, C(4)HCH₃, C(5)H₃], 1.79–1.91 [1H, m, C(4)H], 2.60 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 9.5, one of C(2)H₂], 2.72–2.92 [2H, m, BX of ABX, one of C(2)H₂, C(3)H], 7.10–7.28 (5H, m, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 50 mL) and 4-methyl-3-phenylpentanoyl chloride (±)-**2c** (1.00 g, 4.75 mmol) to give the acid (±)-**1c** (900 mg, 99%) as a cream, white solid, m.p. 48-50 °C (Lit.,²⁸ 46-48 °C) and with spectral characteristics identical to those described above.

(±)-4,4-Dimethyl-3-phenylpentanoic acid (±)-1d^{28;31}

This was prepared following the procedure described for (±)-**1b**, from *t*-butyl magnesium chloride (2 M in diethyl ether, 106 mL, 212 mmol) and cinnamic acid (7.85 g, 52.98 mmol) to give the crude acid (±)-**1d** (8.63 g, 79%) as a yellow solid, which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (KBr) 2955 (OH), 1726 (CO), 1638, 1453; δ_{H} (400 MHz) 0.87 [9H, s, C(CH₃)₃], 2.73 [1H, dd, A of ABX, J_{AB} 15.8, J_{AX} 10.8, one of C(2)H₂], 2.81 [1H, dd, B of ABX, J_{AB} 15.8, J_{BX} 4.5, one of C(2)H₂], 2.93 [1H, dd, X of ABX, J_{AX} 10.8, J_{BX} 4.5, C(3)H], 7.07–7.33 (5H, m, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 8 mL) and 4,4-dimethyl-3-phenylpentanoyl chloride (±)-**2d** (214 mg, 0.95 mmol) to give the acid (±)-**1d** (153 mg, 78%) as a white solid, m.p. 108-110 °C (Lit.,²⁸ 114–116 °C) and with spectral characteristics identical to those described above.

(±)-3-(4-Methylphenyl)butanoic acid (±)-1e⁴²

This was prepared from *p*-tolyl magnesium bromide [freshly prepared from magnesium (10 g, 411 mmol) and iodine (catalytic amount) in diethyl ether (80 mL), and 4-bromotoluene (51.6 mL, 419 mmol) in diethyl ether (60 mL)] and crotonic acid (12.00 g, 139 mmol). The reaction mixture, containing the product (±)-**1e** and the Wurtz coupling product, was acidified to pH 2 and the aqueous layer washed with diethyl ether (2 × 100 mL). The combined diethyl ether extracts were washed with sodium hydroxide (20%, 2 × 100 mL) the aqueous layer was acidified to pH 1 with conc. hydrochloric acid and extracted with diethyl ether (3 × 100 mL). The organic layer was dried, filtered and concentrated under reduced pressure to give the crude acid (±)-**1e** as a viscous yellow oil (21.19 g, 86%) which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2926 (OH), 1704 (CO), 1515, 1455, 1416; δ_{H} (300 MHz) 1.30 [3H, d, J 7.0, C(4)H₃], 2.31 [3H, s, C(4')CH₃], 2.55 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 8.2, one of C(2)H₂], 2.65 [1H, dd, B of ABX, J_{AB} 15.5, J_{BX} 6.9, one of C(2)H₂], 3.18-3.30 [1H, m, X of ABX, C(3)H], 7.11 (4H, s, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 8 mL) and 3-(4-methylphenyl)butanoyl acid chloride (±)-**2e** (200 mg, 1.02 mmol) to give the acid (±)-**1e** (152 mg, 83%) as a white solid, m.p. 91-92 °C (Lit.,⁴³ 87-88 °C) and with spectral characteristics identical to those described above.

(±)-3-(3-Methylphenyl)butanoic acid (±)-1f⁴⁴

This was prepared following the procedure described for (±)-**1e** from 3-tolyl magnesium bromide [freshly prepared from magnesium (4.24 g, 174 mmol) and iodine (catalytic amount) in diethyl ether (40 mL), and 3-bromotoluene (21 mL, 173 mmol) in diethyl ether (40 mL)] and crotonic acid (5.00 g, 58 mmol) to give the crude acid (±)-**1f** (8.55 g, 83%) as an orange oil, which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2971 (OH), 1718 (CO), 1608, 1490, 1455; δ_{H} (300 MHz) 1.30 [3H, d, J 7.0, C(4)H₃], 2.33 [3H, s, C(3')CH₃], 2.55 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 8.4, one of C(2)H₂], 2.66 [1H, dd, B of ABX, J_{AB} 15.5, J_{BX} 6.7, one of C(2)H₂], 3.14-3.31 [1H, m, X of ABX, C(3)H], 6.91–7.22 (4H, m, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 150 mL) and 3-(3-methylphenyl)butanoyl chloride (±)-**2f** (4.59 g, 23.36 mmol) to give the acid (±)-**1f** (2.87 g, 69%) as a clear oil and with spectral characteristics identical to those described above.

(±)-3-(2-Methylphenyl)butanoic acid (±)-1g⁴⁵

This was prepared following the procedure described for (±)-**1e** from 2-tolyl magnesium bromide [freshly prepared from magnesium (4.22 g, 174 mmol) and iodine (catalytic amount) in diethyl ether (40 mL), and 2-bromotoluene (21 mL, 175 mmol) in diethyl ether (40 mL)] and crotonic acid (5.00 g, 58 mmol) to give the crude acid (±)-**1g** (8.21 g, 79%) as an orange oil, which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2973 (OH), 1712 (CO), 1605, 1492, 1460; δ_{H} (400 MHz) 1.27 [3H, d, J 6.9, C(4)H₃], 2.36 [3H, s, C(2')CH₃], 2.55 [1H, dd, A of ABX, J_{AB} 15.7, J_{AX} 8.6, one of C(2)H₂], 2.67 [1H, dd, B of ABX, J_{AB} 15.6, J_{BX} 6.3, one of C(2)H₂], 3.45-3.58 [1H, m, X of ABX, C(3)H], 7.06–7.27 (4H, m, ArH).

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 100 mL) and 3-(2-methylphenyl)butanoyl chloride (±)-**2g** (3.10 g, 15.75 mmol) to give the acid (±)-**1g** (2.20 g, 78%) as a yellow solid, m.p. 46-48 °C (Lit.,⁴⁵ 46-47 °C) and with spectral characteristics identical to those described above.

(±)-2-(4-Methoxyphenyl)butanoic acid (±)-1h^{46;47}

This was prepared following the procedure described for (±)-**1e** from *p*-methoxyphenyl magnesium bromide [freshly prepared from magnesium (10.22 g, 420 mmol) and iodine (catalytic amount) in diethyl ether (80 mL), and 4-bromoanisole (52 mL, 415 mmol) in diethyl ether (100 mL)] and crotonic acid (12.00 g, 139 mmol) to give the crude acid (±)-**1h** (19.17 g, 71%) as a yellow oil, which was used without further purification. $\nu_{\max}/\text{cm}^{-1}$ (film) 2963 (OH), 1711 (CO), 1611, 1511, 1458; δ_{H} (400 MHz) 1.29 [3H, d, J 7.0, C(4)H₃], 2.54 [1H, dd, A of ABX, J_{AB} 15.4, J_{AX} 8.1, one of C(2)H₂], 2.62 [1H, dd, B of ABX, J_{AB} 15.4, J_{BX} 7.0, one of C(2)H₂], 3.17-3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH₃), 6.79-6.88 [2H, m, C(3')H, C(5')H], 7.11-7.16 [2H, m, C(2')H, C(6')H].

Preparation of the analytically pure acid by basic hydrolysis of the corresponding acid chloride.

This was prepared following the procedure described for (±)-**1b**, from aqueous potassium hydroxide (20%, 8 mL) and 3-(4-methoxyphenyl)butanoyl chloride (±)-**2h** (200 mg, 0.94 mmol) to give the acid (±)-**1h** (114 mg, 62%) as a cream solid, m.p. 66–68 °C (Lit.,⁴⁶ 67–69 °C) and with spectral characteristics identical to those described above.

Synthesis of Acid Chlorides

The acid chlorides were generally prepared from crude acids. The yield given below is the yield of acid chloride over two steps calculated from the unsaturated precursor for the acid.

(±)-3-Phenylpentanoyl chloride (±)-**2b**²⁸

3-Phenylpentanoic acid (±)-**1b** (5.01 g, 28.13 mmol) in thionyl chloride (16 mL, 225 mmol) was heated under reflux for 3 h while stirring under nitrogen. Excess thionyl chloride was evaporated under reduced pressure to give the acid chloride (±)-**2b** as a brown oil. Purification by vacuum distillation gave the acid chloride (±)-**2b** (2.07 g, 31%) as a bright yellow oil; b.p. 72–76 °C at 0.09 mmHg (Lit.,²⁸ 113–115 °C at 0.5 mmHg); $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1799 (CO), 1604, 1495, 1454; δ_{H} (400 MHz) 0.80 [3H, t, J 7.4, C(5)H₃], 1.58–1.80 [2H, m, C(4)H₂], 3.04–3.23 [3H, m, C(2)H₂, C(3)H], 7.16–7.34 (5H, m, ArH).

(±)-4-Methyl-3-phenylpentanoyl chloride (±)-**2c**²⁸

This was prepared following the procedure described for (±)-**2b**, from crude 4-methyl-3-phenylpentanoic acid (±)-**1c** (5.28 g, 27.46 mmol) and thionyl chloride (16 mL, 220 mmol) to give the crude acid chloride (±)-**2c** as a brown oil. Purification by distillation gave the acid chloride (±)-**2c** (2.54 g, 36%) as a clear oil; b.p. 78–80 °C at 0.15 mmHg (Lit.,²⁸ 90–94 °C at 0.08 mmHg); $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1799 (CO), 1602, 1495, 1454; δ_{H} (400 MHz) 0.76, 0.97 [2 × CH₃, 2 × d, J 6.7, J 6.7, C(4)HCH₃, C(5)H₃], 1.82–1.94 [1H, m, C(4)H], 2.92–2.98 [1H, m, X of ABX, C(3)H], 3.18 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 9.7, one of C(2)H₂], 3.34 [1H, dd, B of ABX, J_{AB} 16.4, J_{AX} 5.2, one of C(2)H₂], 7.09–7.35 (5H, m, ArH).

(±)-4,4-Dimethyl-3-phenylpentanoyl chloride (±)-**2d**²⁸

This was prepared following the procedure described for (±)-**2b**, from crude 4,4-dimethyl-3-phenylpentanoic acid (±)-**1d** (8.63 g, 42 mmol) and thionyl chloride (24 mL, 335 mmol) to give the crude acid chloride (±)-**2d** as a brown oil. Purification by distillation gave the acid chloride (±)-**2d** (5.04 g, 54%) as a bright yellow solid, b.p. 84–86 °C at 0.12 mmHg (Lit.,²⁸ 123–125 °C at 0.1 mmHg); $\nu_{\text{max}}/\text{cm}^{-1}$ (KBr) 1793 (CO), 1601, 1494, 1453; δ_{H} (400 MHz) 0.91 [9H, s, C(CH₃)₃], 3.04 [1H, dd, X of ABX, J 8.9, 6.1, C(3)H], 3.26–3.39 [2H, m, C(2)H₂], 7.14–7.31 (5H, m, ArH).

(±)-3-(4-Methylphenyl)butanoyl chloride (±)-2e⁴⁸

This was prepared following the procedure described for (±)-**2b**, from crude 3-(4-methylphenyl)butanoic acid (±)-**1e** (21.19 g, 119 mmol) and thionyl chloride (86 mL, 1190 mmol) to give the crude acid chloride (±)-**2e** as a black oil. Purification by distillation gave the acid chloride (±)-**2e** (12.09 g, 44%) as a dark orange oil, b.p. 66–68 °C at 0.2 mmHg (Lit.,⁴⁸ 127 °C at 0.20 mmHg); $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1800 (CO), 1649, 1516, 1455; δ_{H} (300 MHz) 1.32 [3H, d, J 7.0, C(4)H₃], 2.32 [3H, s, C(4')CH₃], 3.07 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 7.9, one of C(2)H₂], 3.17 [1H, dd, B of ABX, J_{AB} 16.4, J_{BX} 6.4, one of C(2)H₂], 3.27–3.38 [1H, m, X of ABX, C(3)H], 7.04–7.21 (4H, m, ArH).

(±)-3-(3-Methylphenyl)butanoyl chloride (±)-2f

This was prepared following the procedure described for (±)-**2b**, from crude 3-(3-methylphenyl)butanoic acid (±)-**1f** (8.55 g, 47.97 mmol) and thionyl chloride (28 mL, 384 mmol) to give the crude acid chloride (±)-**2f** as a black oil. Purification by distillation gave the acid chloride (±)-**2f** (4.59 g, 40%) as a bright yellow oil, b.p. 77–80 °C at 0.2 mmHg; $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1800 (CO), 1608, 1491, 1456; δ_{H} (300 MHz) 1.32 [3H, d, J 6.9, C(4)H₃], 2.33 [3H, s, C(3')CH₃], 3.06 [1H, dd, A of ABX, J_{AB} 16.5, J_{AX} 8.0, one of C(2)H₂], 3.17 [1H, dd, B of ABX, J_{AB} 16.5, J_{BX} 6.5, one of C(2)H₂], 3.25–3.36 [1H, m, X of ABX, C(3)H], 6.98–7.05 [3H, m, C(4')H, C(5')H, C(6')H, ArH], 7.17–7.22 [1H, m, C(2')H, ArH].

(±)-3-(2-Methylphenyl)butanoyl chloride (±)-2g

This was prepared following the procedure described for (±)-**2b**, from crude 3-(2-methylphenyl)butanoic acid (±)-**1g** (8.21 g, 46 mmol) and thionyl chloride (27 mL, 368 mmol) to give the crude acid chloride (±)-**2g** as a black oil. Purification by distillation gave the acid chloride (±)-**2g** (4.68 g, 41%) as a bright yellow oil, b.p. 64–66 °C at 0.09 mmHg; $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1801 (CO), 1605, 1492, 1459; δ_{H} (400 MHz) 1.27 [3H, d, J 7.0, C(4)H₃], 2.35 [3H, s, C(2')CH₃], 3.03 [1H, dd, A of ABX, J_{AB} 16.6, J_{AX} 8.2, one of C(2)H₂], 3.16 [1H, dd, B of ABX, J_{AB} 16.6, J_{BX} 6.3, one of C(2)H₂], 3.54–3.63 [1H, m, X of ABX, C(3)H], 7.07–7.28 (4H, m, ArH).

(±)-3-(4-Methoxyphenyl)butanoyl chloride (±)-2h⁴⁶

This was prepared following the procedure described for (±)-**2b**, from crude 3-(4-methoxyphenyl)butanoic acid (±)-**1h** (19.17 g, 99 mmol), thionyl chloride (57 mL, 790 mmol) to give the crude acid chloride (±)-**2h** as a black oil. Purification by distillation gave the acid chloride (±)-**2h** (8.69 g, 29%) as a orange brown oil, b.p. 102–110 °C at 0.35 mmHg (Lit.,⁴⁶ 100 °C at 0.5 mmHg); $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 1790 (CO), 1614, 1515, 1463; δ_{H} (400 MHz) 1.32 [3H, d, J 7.0, C(4)H₃], 3.07 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 7.8, one of C(2)H₂], 3.15 [1H, dd, B of ABX, J_{AB} 16.4, J_{BX} 6.8, one of C(2)H₂], 3.27–3.36 [1H, m, X of ABX, C(3)H], 3.78 (3H, s, OCH₃), 6.84–6.87 [2H, m, C(3')H, C(5')H], 7.11–7.15 [2H, m, C(2')H, C(6')H].

Synthesis of Ethyl Esters

(±)-Ethyl 3-phenylbutanoate (±)-**3a**⁴⁹

Sulfuric acid (1.0 mL, 18.76 mmol) was added to a solution of 3-phenylbutanoic acid (±)-**1a** (998 mg, 6.08 mmol) in absolute ethanol (20 mL) and refluxed overnight. Excess ethanol was evaporated under reduced pressure. The crude product was dissolved in dichloromethane (45 mL) and washed with water (2 × 45 mL), sat. NaHCO₃ (2 × 45 mL), brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude ester (±)-**3a** (972 mg) as a clear oil. Purification by chromatography on silica gel using hexane/ethyl acetate 60/40 as eluant gave the pure ester (±)-**3a** (909 mg, 78%) as a clear oil; $\nu_{\max}/\text{cm}^{-1}$ (film) 2969 (CH), 1733 (CO), 1604, 1495, 1454 (Ar), 1174 (C-O); δ_{H} (400 MHz) 1.17 (3H, t, J 7.1, OCH₂CH₃), 1.30 [3H, d, J 7.0, C(4)H₃], 2.53 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H₂], 2.61 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.0, one of C(2)H₂], 3.23-3.32 [1H, sym. m, X of ABX, C(3)H], 4.08 (2H, q, J 7.1, OCH₂CH₃), 7.17–7.34 (5H, m, ArH).

(±)-Ethyl 3-phenylpentanoate (±)-**3b**⁵⁰

3-Phenylpentanoyl chloride (±)-**2b** (1.96 g, 9.96 mmol) in dichloromethane (10 mL) was added dropwise to a solution of triethylamine (1.7 mL, 11.9 mmol), dichloromethane (10 mL) and distilled ethanol (1.45 mL, 24.9 mmol), at 0 °C. The reaction mixture was stirred at room temperature overnight. The crude product was dissolved in dichloromethane (30 mL) and washed with water (2 × 50 mL), HCl (10%, 2 × 50 mL), brine (100 mL) dried, filtered and concentrated under reduced pressure to give the crude ester (±)-**3b** (1.64 g) as a deep orange oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the pure ester (±)-**3b** (1.49 g, 72%) as a clear oil; $\nu_{\max}/\text{cm}^{-1}$ (film) 2967 (CH), 1735 (CO), 1603, 1493, 1454 (Ar), 1167 (C-O); δ_{H} (400 MHz) 0.79 [3H, t, J 7.4, C(5)H₃], 1.13 (3H, t, J 7.1, OCH₂CH₃), 1.55-1.76 [2H, m, C(4)H₂], 2.55 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H₂], 2.63 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.1, one of C(2)H₂], 2.96-3.04 [1H, m, X of ABX, C(3)H], 4.02 (2H, q, J 7.1, OCH₂CH₃), 7.17-7.30 (5H, m, ArH).

(±)-Ethyl 4-methyl-3-phenylpentanoate (±)-**3c**⁵¹

This was prepared following the procedure described for (±)-**3b**, from 4-methyl-3-phenylpentanoyl chloride (±)-**2c** (2.43 g, 11.52 mmol), triethylamine (1.9 mL, 13.82 mmol), dichloromethane (10 mL) and distilled ethanol (1.7 mL, 28.79 mmol) to yield the crude ester (±)-**3c** (2.02 g) as a yellow orange oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the pure ester (±)-**3c** (1.6 g, 63%) as a clear oil. $\nu_{\max}/\text{cm}^{-1}$ (film) 2961 (CH), 1736 (CO), 1602, 1494, 1453 (Ar), 1162 (C-O); δ_{H} (400 MHz) 0.75, 0.95 [2 × 3H, 2 × d, J 6.7, J 6.7, C(4)HCH₃, C(5)H₃], 1.05 (3H, t, J 7.1, OCH₂CH₃), 1.79–1.91 [1H, sym m, C(4)H], 2.58 [1H, dd, A of ABX, J_{AB} 14.9, J_{AX} 9.9, one of C(2)H₂], 2.77 [1H, dd, B of ABX, J_{AB} 14.9, J_{BX} 5.6, one of C(2)H₂], 2.85–2.91 [1H, m, X of ABX, C(3)H], 3.95 (2H, q, J 7.1, OCH₂CH₃), 7.14–7.28 (5H, m, ArH).

(±)-Ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**3d**⁵²

This was prepared following the procedure described for (±)-**3b**, from 4,4-dimethyl-3-phenylpentanoyl chloride (±)-**2d** (5.04 g, 22.4 mmol), triethylamine (3.8 mL, 26.9 mmol), dichloromethane (30 mL) and distilled ethanol (3.3 mL, 56.1 mmol) to yield the crude ester

(±)-**3d** (3.81 g) as a bright yellow oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the pure ester (±)-**3d** (2.94 g, 56%) as pale yellow oil. $\nu_{\max}/\text{cm}^{-1}$ (film) 2965 (CH), 1737 (CO), 1602, 1454 (Ar), 1152 (C-O); δ_{H} (400 MHz) 0.89 [9H, s, C(CH₃)₃], 0.99 (3H, t, J 7.1 OCH₂CH₃), 2.71 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 10.9, one of C(2)H₂], 2.79 [1H, dd, B of ABX, J_{AB} 15.2, J_{BX} 5.0, one of C(2)H₂], 2.98 [1H, dd, X of ABX, J_{AX} 10.9, J_{BX} 5.0, C(3)H], 3.85–3.97 (2H, m, OCH₂CH₃), 7.13–7.26 (5H, m, ArH).

(±)-Ethyl 3-(4-methylphenyl)butanoate (±)-**3e**²⁴

This was prepared following the procedure described for (±)-**3b**, from 3-(4-methylphenyl)butanoyl chloride (±)-**2e** (12.09 g, 61.47 mmol), triethylamine (10.28 mL, 73.76 mmol), dichloromethane (25 mL) and distilled ethanol (8.9 mL, 153.7 mmol) to yield the crude ester (±)-**3e** (9.41 g) as a deep orange oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the pure ester (±)-**3e** (7.84 g, 62%) as a clear oil. $\nu_{\max}/\text{cm}^{-1}$ (film) 2967 (CH), 1732 (CO), 1516, 1456 (Ar), 1166 (C-O); δ_{H} (400 MHz) 1.19 (3H, t, J 7.1, OCH₂CH₃), 1.28 [3H, d, J 7.0, C(4)H₃], 2.32 [3H, s, C(4')CH₃], 2.51 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H₂], 2.59 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.0, one of C(2)H₂], 3.20–3.29 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, J 7.2, OCH₂CH₃), 7.11 (4H, s, ArH).

(±)-Ethyl 3-(3-methylphenyl)butanoate (±)-**3f**

This was prepared following the procedure described for (±)-**3b**, from 3-(3-methylphenyl)butanoyl chloride (±)-**2f** (7.32 g, 37.2 mmol), triethylamine (6.2 mL, 44.64 mmol), dichloromethane (30 mL) and distilled ethanol (5.4 mL, 92.99 mmol) to yield the crude ester (±)-**3f** (6.53 g) as a dark brown oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the pure ester (±)-**3f** (5.16 g, 67%) as a clear oil. (Found C, 75.13; H 8.77. C₁₃H₁₈O₂ requires C, 75.69; H, 8.80%); $\nu_{\max}/\text{cm}^{-1}$ (film) 2968 (CH), 1733 (CO), 1608, 1461 (Ar), 1177 (C-O); δ_{H} (400 MHz) 1.18 (3H, t, J 7.1, OCH₂CH₃), 1.28 [3H, d, J 7.0, C(4)H₃], 2.32 [3H, s, C(3')CH₃], 2.51 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.3, one of C(2)H₂], 2.59 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 6.9, one of C(2)H₂], 3.19–3.28 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, J 7.1, OCH₂CH₃), 6.97–7.05 [3H, m, C(4')H, C(5')H, C(6')H, ArH], 7.13–7.19 [1H, m, C(2')H, ArH]. δ_{C} (75.5 MHz) 14.2, 21.5, 21.8 [3 x CH₃, C(4)H₃, OCH₂CH₃, C(3')CH₃], 36.5 [CH, C(3)H], 43.0 [CH₂, C(2)H₂], 60.3 [CH₂, OCH₂CH₃], 123.7, 127.1, 127.6, 128.4 (4 x CH, aromatic CH), 138.0, 145.8 (2 x C, aromatic C), 172.5 [C, C(1)]. HRMS (ES⁺): Exact mass calculated for C₁₃H₁₈O₂ [M+H]⁺ 207.1385. Found 207.1390; m/z (ES⁺) 207.1 {[(C₁₃H₁₈O₂)+H]⁺, 57%}, 202.1 (100%), 161.1 {[(C₁₁H₁₃O)-C₂H₅O]⁺, 60%}, 151.0 (23%), 141.0 (7%).

(±)-Ethyl 3-(2-methylphenyl)butanoate (±)-**3g**

This was prepared following the procedure described for (±)-**3b**, from 3-(2-methylphenyl)butanoyl chloride (±)-**2g** (4.68 g, 23.79 mmol), triethylamine (4 mL, 28.54 mmol), dichloromethane (20 mL) and distilled ethanol (3.5 mL, 59.46 mmol) to yield the crude ester (±)-**3g** (2.85 g) as a yellow oil. Purification by chromatography on silica gel using hexane/ether 97/3 as eluant gave the ester (±)-**3g** (2.5 g, 67%) as a clear oil. Further purification by distillation gave the ester (±)-**3g** (2.14 g, 44%) as a clear oil, b.p. 96°C at 0.06 mmHg; (Found C, 75.43; H 8.67. C₁₃H₁₈O₂ requires C, 75.69; H, 8.80%); $\nu_{\max}/\text{cm}^{-1}$ (film)

2975 (CH), 1733 (CO), 1605, 1492, 1462 (Ar), 1175 (CO); δ_{H} (400 MHz) 1.17 (3H, t, J 7.1, OCH_2CH_3), 1.25 [3H, d, J 6.9, C(4) H_3], 2.37 [3H, s, C(2') CH_3], 2.52 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 8.5, one of C(2) H_2], 2.61 [1H, dd, B of ABX, J_{AB} 15.2, J_{BX} 6.6, one of C(2) H_2], 3.46–3.61 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, J 7.1, OCH_2CH_3), 7.07–7.19 (4H, m, ArH). δ_{C} (75.5 MHz) 14.2, 19.5, 21.3 [3 x CH_3 , C(4) H_3 , OCH_2CH_3 , C(2') CH_3], 31.5 [CH, C(3)H], 42.2 [CH_2 , C(2) H_2], 60.3 [CH_2 , OCH_2CH_3], 125.1, 126.1, 126.3, 130.4 (4 x CH, aromatic CH), 135.3, 143.9 (2 x C, aromatic C), 172.6 [C, C(1)]. HRMS (ES⁺): Exact mass calculated for $\text{C}_{13}\text{H}_{18}\text{O}_2$ $[\text{M}+\text{H}]^+$ 207.1385. Found 207.1393; m/z (ES⁺) 207.1 $\{[(\text{C}_{13}\text{H}_{18}\text{O}_2)+\text{H}]^+, 71\%\}$, 202.1 (100%), 161.1 $\{[(\text{C}_{11}\text{H}_{13}\text{O})-\text{C}_2\text{H}_5\text{O}]^-, 48\%\}$, 151.0 (68%), 141.0 (24%).

(±)-Ethyl 3-(4-methoxyphenyl)butanoate (±)-**3h**⁵³

This was prepared following the procedure described for (±)-**3b**, from 3-(4-methoxyphenyl)butanoyl chloride (±)-**2h** (8.49 g, 39.9 mmol), triethylamine (6.7 mL, 47.88 mmol), dichloromethane (50 mL) and distilled ethanol (5.8 mL, 99.75 mmol) to yield the crude ester (±)-**3h** (6.68 g) as an orange oil. Purification by chromatography on silica gel using hexane/ethyl acetate as eluant (gradient elution 0–10% ethyl acetate) gave the ester (±)-**3h** (5.32 g, 56%) as a clear oil. $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 2966 (CH), 1732 (CO), 1613, 1513, 1461 (Ar), 1174 (CO); δ_{H} (400 MHz) 1.18 (3H, t, J 7.1, OCH_2CH_3), 1.27 [3H, d, J 7.0, C(4) H_3], 2.5 [1H, dd, A of ABX, J_{AB} 14.9, J_{AX} 8.0, one of C(2) H_2], 2.57 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.2, one of C(2) H_2], 3.17–3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH_3), 4.07 (2H, q, J 7.1, OCH_2CH_3), 6.81–6.85 [2H, m, C(3')H, C(5')H], 7.12–7.16 [2H, m, C(2')H, C(6')H].

Enzyme Screening

General procedure for the hydrolase catalysed kinetic resolution of the 3-aryl alkanolic ethyl esters (±)-**3a-i** (analytical scale).

A spatula tip of enzyme (~5–10 mg) was added to the substrate (±)-**3a-i** (~50 mg) in 0.1 M phosphate buffer, pH 7 (4.5 mL). Co-solvents (17% v/v) were added as indicated in Table 3. The reaction vessel was shaken at 700–750 rpm and incubated at the appropriate temperature for the required length of time. The aqueous layer was extracted with diethyl ether (3 x 5 mL) and the combined organic extracts were filtered through celite and concentrated under reduced pressure. The sample was analysed by ^1H NMR spectroscopy, reconcentrated and dissolved in a mixture of hexane:*iso*-propyl alcohol (HPLC grade) and enantioselectivity determined by chiral HPLC. The results of the screen are summarised in the appropriate Tables 1–10.

Preparative scale hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-**3a**

Pseudomonas fluorescens (108 mg) was added to ethyl 3-phenylbutanoate (±)-**3a** (510 mg, 2.65 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL) and this was shaken at 750 rpm for 64 h at 30 °C. The solution was filtered through a pad of celite and the hydrolase washed with water (2 x 20 mL) and ethyl acetate (10 x 10 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 30 mL) and then acidified with 10% HCl solution and extracted with a further (3 x 30 mL) ethyl acetate. The combined organic layers

were washed with brine (1 × 100 mL) dried, filtered and concentrated under reduced pressure to produce a clear oil (395 mg). Conversion estimated at 50%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3a** (178 mg, 35%) as a clear oil $[\alpha]_D^{20}$ -27.55 (c 1.1, CHCl₃), 99% ee, lit⁵⁴ $[\alpha]_D^{25}$ +19.00 (c 1.1, CHCl₃), (*S*)-isomer, 90% ee and acid (*S*)-**1a** (147 mg, 34%) as a clear oil $[\alpha]_D^{20}$ +27.90 (c 1.0, EtOH), 98% ee, lit⁵⁵ $[\alpha]_D^{25}$ +24.50 (c 1.0, EtOH), 97% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-3b

Candida antarctica lipase B (immob) (410 mg) was added to ethyl 3-phenylpentanoate (±)-**3b** (408 mg, 1.98 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL) and dioxane (17% v/v, 4 mL). The reaction mixture was shaken at 750 rpm for 62 h at 30 °C, the solution was filtered through a pad of celite and the hydrolase washed with water (2 × 20 mL) and heptane (10 × 10 mL). The layers were separated and the aqueous layer was extracted with heptane (3 × 30 mL). The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the ester (*S*)-**3b** (79.2 mg, 19%) as a light yellow oil. $[\alpha]_D^{20}$ +9.455 (c 0.55, CHCl₃), 65% ee, lit⁵⁶ $[\alpha]_D^{26}$ -18.3 (c 1.1, CHCl₃), (*R*)-isomer, 97% ee. The aqueous layer was acidified with 10% HCl solution and extracted with (3 × 30 mL) ethyl acetate. The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the acid (*R*)-**1b** (77.6 mg, 22%) as a yellow oil. $[\alpha]_D^{20}$ -33.73 (c 1.37, C₆H₆), 90% ee, lit³⁹ $[\alpha]_D^{25}$ +42.3 (c 8.0, C₆H₆), (*S*)-isomer, 83% ee. Conversion estimated at 42%.³⁴ ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 4-methyl-3-phenylpentanoate (±)-3c

This was prepared following the procedure described for (±)-**3b** from *Candida antarctica* lipase B (immob) (426 mg) and ethyl 4-methyl-3-phenylpentanoate (±)-**3c** (428 mg, 1.94 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 63 h at 30 °C to produce the ester (*R*)-**3c** (107 mg, 25%) as a clear oil $[\alpha]_D^{20}$ +7.05 (c 1.0, CHCl₃), 26% ee, lit⁵⁶ $[\alpha]_D^{26}$ -25.4 (c 1.0, CHCl₃), (*S*)-isomer, 98% ee, and the acid (*S*)-**1c** (88 mg, 24%) as a clear oil $[\alpha]_D^{20}$ -24.35 (c 0.655, CHCl₃), 98% ee, lit⁵⁷ $[\alpha]_D^{23}$ +28.12 (c 1.855, CHCl₃), (*R*)-isomer, 96% ee. Conversion estimated at 21%.³⁴ ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-3d

This was prepared following the procedure described for (±)-**3b** from *Candida antarctica* lipase B (immob) (200 mg) and ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**3d** (200 mg, 0.85 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for ~65 h at 35 °C and at 40 °C for the final 24 h to produce the ester (*R*)-**3d** (79 mg, 39%) as a clear oil. $[\alpha]_D^{20}$ +8.00 (c 1.0, CHCl₃), 12% ee and the acid (*S*)-**1d** (23 mg, 13%) as a yellow oil which solidified overnight $[\alpha]_D^{20}$ -10.53 (c 0.114, CHCl₃), 99% ee, lit⁵⁸ $[\alpha]_D^{20}$ -20.4 (c 2.2, CHCl₃), 91% ee. Conversion estimated at 11%.³⁴ ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 3-(4-methylphenyl)butanoate (±)-3e

This was prepared following the procedure described for (±)-**1a** from *Pseudomonas cepacia* P1 (95 mg) and ethyl 3-(4-methylphenyl)butanoate (±)-**3e** (446 mg, 2.26 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 62 h at 30 °C to produce a yellow oil (361 mg). Conversion estimated at 49%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3e** (145 mg, 31%) as a clear oil $[\alpha]_D^{20}$ -28.67 (c 3.5, CHCl₃), 97% ee, lit²⁴ $[\alpha]_D^{25}$ -26.2 (c 3.5, CHCl₃), 92% ee and acid (*S*)-**1e** (163 mg, 40%) as a yellow oil which solidified overnight $[\alpha]_D^{20}$ +31.80 (c 1.0, CHCl₃), ≥99% ee, lit²⁴ $[\alpha]_D^{25}$ +34.2 (c 1.0, CHCl₃), 99% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of ethyl 3-(3-methylphenyl)butanoate (±)-3f

This was prepared following the procedure described for (±)-**3a** from *Pseudomonas fluorescens* (94 mg) and ethyl 3-(3-methylphenyl)butanoate (±)-**3f** (471 mg, 2.28 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 62 h at 30 °C to produce a yellow oil (232 mg). Conversion estimated at 49%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3f** (105 mg, 22%) as a yellow oil $[\alpha]_D^{20}$ -24.40 (c 1.0, CHCl₃), 94% ee and acid (*S*)-**1f** (107 mg, 26%) as a clear oil $[\alpha]_D^{20}$ +32.32 (c 0.622, CHCl₃), ≥99% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of ethyl 3-(2-methylphenyl)butanoate (±)-3g

This was prepared following the procedure described for (±)-**1a** from *Pseudomonas fluorescens* (74 mg) and ethyl 3-(2-methylphenyl)butanoate (±)-**3g** (371 mg, 1.80 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 66 h at 30 °C to produce a yellow oil (268 mg). Conversion estimated at 50%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3g** (100 mg, 27%) as a clear oil $[\alpha]_D^{20}$ -11.00 (c 1.0, CHCl₃), 98% ee and acid (*S*)-**1g** (90 mg, 28%) as a yellow oil $[\alpha]_D^{20}$ +24.17 (c 1.38, CHCl₃), ≥99% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-3h

This was prepared following the procedure described for (±)-**1a** from *Pseudomonas fluorescens* (100 mg) and ethyl 3-(4-methoxyphenyl)butanoate (±)-**3h** (498 mg, 2.24 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 64 h at 30 °C to produce an orange oil (428 mg). Conversion estimated at 51%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3h** (212 mg, 43%) as a clear oil $[\alpha]_D^{20}$ -30.03 (c 1.034, CHCl₃), 99% ee and acid (*S*)-**1h** (99 mg, 23%) as a yellow oil $[\alpha]_D^{20}$ +26.25 (c 1.0, EtOH), 97% ee, lit⁵⁵ $[\alpha]_D^{25}$ +27.50 (c 1.0, EtOH) 94% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

Preparative scale hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-3i

This was prepared following the procedure described for (±)-**1a** from *Pseudomonas fluorescens* (45 mg) and ethyl 3-(4-fluorophenyl)butanoate (±)-**3i** (221 mg, 1.05 mmol) in 0.1

M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 64 h at 30 °C to produce a yellow oil (171 mg). Conversion estimated at 51%.³⁴ Purification by column chromatography using hexane/ethyl acetate as eluant (gradient elution 10-40% ethyl acetate) gave ester (*R*)-**3i** (71 mg, 32%) as a clear oil $[\alpha]_D^{20}$ -24.34 (c 1.0, CHCl₃), $\geq 99\%$ ee, and acid (*S*)-**1i** (67 mg, 35%) as a brown oil $[\alpha]_D^{20}$ +30.51 (c 1.016, CHCl₃), 97% ee. ¹H NMR spectra were identical to the racemic materials previously prepared.

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